

Evaluation of the effect on heart rate variability of a β_2 -adrenoceptor agonist and antagonist using non-linear scatterplot and sequence methods

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Aims To examine the impact on heart rate variability (HRV), of agonism or antagonism at the cardiac β_2 -adrenoceptor in healthy volunteers, using standard time-domain summary statistics and non-linear methods (scatterplot and quadrant analysis).

Methods Under double-blind and randomised conditions (Latin square design), 17 normal volunteers received placebo, salbutamol (β_2 -adrenoceptor partial agonist), ICI 118,551 (specific β_2 -adrenoceptor antagonist), or salbutamol plus ICI 118,551. Single oral doses of medication (at weekly intervals) were administered at 22.30 h, with HRV assessed from the sleeping heart rates.

Results Salbutamol reduced the long-term (SDNN: 135 ms [120, 156], SDANN: 107 ms [89, 124]) time-domain indicators of HRV compared with placebo (SDNN: 39 [24, 55], SDANN 42 [29, 56], {mean difference [95% confidence intervals of difference]}). Alone, ICI 118,551 did not effect HRV, but in combination blocked the actions of salbutamol. Scatterplot length (944 ms [869, 1019]) and area (222×10^3 ms² [191, 253]) were reduced by salbutamol compared with placebo; (length difference (164 [98, 230]) and area difference 59 [36, 83]). Scatterplot width (dispersion) was lower at both low (width RR-1 25% salbutamol 277 ms [261, 293]: salbutamol minus placebo 14 ms [0, 28]) and high (width 75% salbutamol 417 [391, 443]: salbutamol minus placebo 41 [20, 62]) heart rates. ICI 118,551 alone did not alter scatterplot parameters but in combination blocked the effect of salbutamol. Cardiac acceleration episodes (i.e. consecutive ΔRR and ΔRR_{n+1} shorten) were increased following salbutamol 7288 [6089, 8486] compared with placebo -1890 [-2600, -1179]; the beat-to beat difference (ΔRR_{n+1}) was reduced after salbutamol compared with the other treatments. ICI 118,551 did not effect acceleration episodes but reduced the effect of salbutamol when used in combination.

Conclusions Agonism at the cardiac β_2 -adrenoceptor in healthy volunteers with salbutamol altered autonomic balance towards sympathetic dominance; this re-balancing was blocked by ICI 118,551 given in combination with salbutamol. However antagonism at the β_2 -adrenoceptor with ICI 118,551 alone did not significantly alter the HRV. The β_2 -adrenoceptor modulates HRV in healthy volunteers; the implications of agonism and antagonism at the β_2 -adrenoceptor in cardiovascular disease states warrants further investigation.

Keywords: heart rate variability, scatterplot, non-linear, β -adrenoceptor, partial agonist

Introduction

Heart-rate variability (HRV) is a powerful predictor of outcome in established cardiovascular disease [1–3];

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disturbed autonomic balance contributes to the impaired prognosis [4, 5]. Drugs that oppose cardiac sympathetic stimulation, such as the β -adrenoceptor antagonists, have improved patient survival following myocardial infarction [6, 7]. It is unclear whether secondary prevention benefits result from a re-balancing of the autonomic nervous system; however HRV has been augmented following

β -adrenoceptor antagonism in cardiovascular disease states [8, 9].

The relationship between the ancillary pharmacological properties of a β -adrenoceptor drug [10] and HRV is a neglected area. We have demonstrated that the partial β -adrenoceptor agonist celiprolol reduced time-domain and non-linear measures of heart rate variability; a component of the reduced variability appeared mediated through the β_2 -adrenoceptor [11]. We therefore sought to extend these observations on HRV effects of agonism and antagonism at the β_2 -adrenoceptor in normal volunteers under randomised, double-blind and placebo controlled conditions. The actions of salbutamol (a β_2 -adrenoceptor partial agonist), ICI 118,551 (a specific β_2 -adrenoceptor antagonist) and the combination of salbutamol plus ICI 118,551 have been studied.

HRV may be assessed by different methods; these have been critically evaluated [12]. The time-domain summary statistics provide information on both short and long-term heart rate variations; however, the pattern of these fluctuations is not evident. Spectral methods provide a time-averaged estimate of cyclic variability and are most useful in the assessment of sinusoidal or near-sinusoidal variations in HR [13, 14]. It has been suggested that non-linear phenomena are involved in the genesis of HRV (i.e. the heart rate shows non-linear complexity or 'chaotic' behaviour [15]); analyses based on non-linear dynamics may prove superior and allow more precise outcome prediction [16].

We have therefore, in addition to the standard time-domain statistics, used the Poincaré plot and quadrant methods to determine HRV with non-linear techniques in this study. The Poincaré plot is a 'scatterplot' (return map) of current cycle is plotted against the previous beat (RR vs RR_{n-1}); scatterplot length is correlated with long-term (SDNN and SDANN) time domain statistics [17] while scatterplot width appears a specific measure of parasympathetic nervous system activity [17, 18]. Quadrant analysis is another non-linear method proposed to investigate the nature of cardiac interval sequencing [13]; here the interval difference between two consecutive beats and the next pair is evaluated (ΔRR vs ΔRR_{n+1}). Quadrant analysis removes the dominant characteristics apparent in the Poincaré plot, namely the high correlation between one interval and the next.

Methods

Seventeen healthy male volunteers (age 30 ± 3 years; weight 71 ± 10 kg) were studied. Overnight tape records were retrieved from archive from two placebo-controlled, double-blind, balanced (Latin square design) studies [19, 20]. The original objective of these studies was to evaluate the properties of some β -adrenoceptors, using the sleeping

hourly heart rate as one of the assessment parameters. The effects of agonists of the β -adrenoceptor can most readily be assessed at night, due to the low level of prevailing sympathetic drive and catecholamine levels. The nocturnal parasympathetic dominance results in a progressive fall in the heart rate that reaches a nadir approximately 6 h after onset of sleep; sleeping heart rate is further reduced by β -adrenoceptor antagonists and this contrasts with the effects of partial agonists of the β -adrenoceptor that increase or prevent the normal fall in the sleeping heart rate [21]. When the studies were originally undertaken, it was considered that the limitations imposed by the available technology, precluded detailed HRV analyses.

Among the treatments each volunteer had received (with an interval of at least 1 week), was single oral doses of placebo, salbutamol 8 mg, ICI 118,551 25 mg, or salbutamol 8 mg plus ICI 118,551 25 mg. Medication was administered between 22.15 and 22.45 h. Salbutamol behaves either as a full or partial agonist at the β_2 -adrenoceptor [22]; it is 107 times more selective for the β_2 compared with the β_1 -adrenoceptor (comparison of EC_{50} values for the relative effects of an agonist on β_1 and β_2 mediated responses) [23]. ICI 118,551 possessed a high degree of selectivity and specificity for the β_2 -adrenoceptor; in comparison with propranolol (β_2/β_1 selectivity ratio 2.2), ICI 118,551 had a ratio of 123; it was devoid of partial agonist activity [24]. Thus it was a useful drug for examining the role of the β_2 -adrenoceptor in physiological control mechanisms and in disease states. ICI 118,551 at doses above 50 mg (particularly with chronic dosing) had demonstrable effects on β_1 -adrenoceptors; however at 20 mg there were no, or minimal effects on dobutamine induced positive inotropic responses [25]. Doses of ICI 118,551 of 20 mg orally had minimal effects on peak exercise heart rate or on the BP response to infused isoprenaline in normal volunteers [20], further evidence of its specificity for the β_2 -adrenoceptor. ICI 118,551 25 mg had no effect on the sleeping heart rate [19].

Heart rate methods

The HR was recorded (modified lead II position) using an Oxford Medilog 2–24 miniature analogue tape recorder, with a phase-locked loop motor speed control to ensure speed stability. The recording speed was 2 mm s^{-1} . The Holter tape records were analysed using a neural network classification (Biomedical Systems ColortraceTM—St Louis, MO, 63146, USA). HRV was assessed from RR interval files generated from 22.00 to 08.00 h, analysed in hourly epochs. Aberrant data values were excluded; further filtering used a validated operator independent method for establishing HRV from continuous

extended ECG recordings [26]. This method accepted only consecutive beats of normal morphological characteristics (N-N) with cycle lengths within 17.5% of the preceding cycle length.

Time-domain summary statistics

The summary time-domain statistical measures of HRV calculated included the standard deviation of all NN intervals (SDNN), the standard deviation of the averages of the NN intervals in all 5 min segments of the recording (SDANN), the mean of the standard deviations of all NN intervals for all 5 min segments of the recording (SDNN index), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (rmsSD) and the number of pairs of adjacent NN counts differing by more than 50 ms divided by the total number of all NN intervals (pNN_{50}). These measures have been summarised [12]; SDNN reflects all cyclic components responsible for variability in the recording, SDANN and SDNN index represent variability over cycles longer and shorter than 5 min respectively while rmsSD and pNN_{50} , derived from interval differences, are short-term HRV measures estimating high frequency variations in heart rate.

Scatterplot methods (Figure 1)

Scatterplots were constructed by plotting each RR interval against the preceding RR interval, as previously described [17]. Unlike previous descriptions of the method, where dedicated commercial scanner software produced hard copy of the scatterplots requiring manual measurements, we developed an automated computer method. Scatterplots were initially produced using the charting feature of SPSS (Release 6.1) [27], with its ability to handle large data-sets, and then stored in bitmap

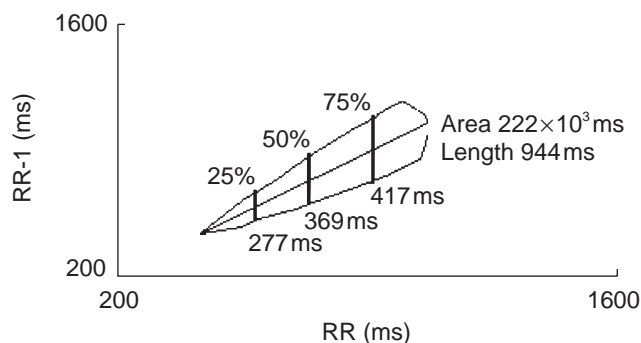


Figure 1 The length of the Poincaré plot (scatterplot) was operator defined. The area was calculated by performing width measurements (10 msec interval) with subsequent rectangular integration. Width measurements were standardised at 10%, 25%, 50%, 75% and 90% of the scatterplot length.

format (Figure 1). A digitising programme (TechDig V2.0a; Ron Jones (ronjones@xnet.com) Mundelein, IL) was customised, to permit automated technician analysis of the Poincaré plot. Initially the bounds of the X and Y axis were defined (reference points and the numeric values for the start and end of each axis entered), using a mouse and fine cross-hairs. The limits of the scatterplot length were defined by clicking on the extremes of the scatterplot diagonal. The computer calculated the scatterplot length (between the two calibration points), width (at 10 ms intervals) and area; these results were available through the WindowsTM clipboard to be pasted into other spreadsheet or data management programmes for statistical processing. The key to the width measurements was a test to determine if a given pixel fell inside or outside the boundaries of the scatterplot. The test counted the coloured pixels in the plot in a 9×21 pixel window centred at the pixel of interest. If more than 20 pixels in this window were coloured, the pixel was considered to be within the scatterplot. The width measurement was made at a given RR interval by performing this test at each pixel along the line perpendicular to the RR axis at the interval of interest. The width was defined by the difference between the first pixel in the scatterplot and the next pixel not in the scatterplot. The area was calculated by performing width measurements at 10 ms intervals and subsequent rectangular integration. Width measurements were also automatically performed at 10%, 25%, 50%, 75% and 90% of the scatterplot length as previously described [28]; the average width of the scatterplot was the measure of variance [17] rather than calculating the variance around the perpendicular to the line of identity (i.e. scatterplot length) [18]. The reproducibility of the method was determined in 12 volunteers, with repeat analysis of a scatterplot on 5 consecutive occasions; the reproducibility for the length, area and width was calculated. The average coefficient of variation (range) was for length 1.0% (0.3–1.8), area 1.3% (0.4–3.1), P10% 3.6% (0–8.9), P25% 3.3% (0–9.0), P50% 1.4% (0–3.3), P 75% 1.3% (0.2–3.8) and P90% 2.0% (0.5–4.9).

Cardiac sequence (quadrant) analysis

The non-linear cardiac sequencing or quadrant method is as previously described [13]. Consecutive ΔRR and ΔRR_{n+1} differences are compared (i.e. three consecutive RR values; two differences $RR_1 - RR_2$ (ΔRR) and $RR_2 - RR_3$: (ΔRR_{n+1})). This method removes the dominant characteristic apparent in comparing each beat with the next—namely the high correlation between one interval and the next. Four patterns can be identified; $+/+$ (a lengthening sequence—cardiac deceleration), $+/-$ or $-/+$ (balanced sequences), and finally $-/-$

(a shortening sequence—cardiac acceleration). Events of each type were counted by quadrant; in addition the average duration (i.e. of the second difference ΔRR_{n+1}) of the difference within each quadrant was calculated. When the ANS is balanced, one would expect the quadrant b/c patterns to predominate (+/- or -/+) in contrast to parasympathetic (quadrant a; +/+) or sympathetic dominance (quadrant d; -/-).

Statistical methods

Tabular summary statistics are presented as mean [95% confidence intervals]. There is some uncertainty about the linear nature of the scaling of HRV parameters, for this reason the overall significance for each variable was calculated using non-parametric two way analysis of variance (Friedman's procedure); where significance was indicated, the data-sets were then examined using the Wilcoxon sign test to determine the between group *P* values [29]. Differences between groups were considered significant at the *P*<0.01 level.

Results

Effects on heart rate (Table 1: Figure 2)

Over the course of the night the sleeping heart rate decreased significantly. Commencing 2 h post-dosing differences between the drugs were apparent; between 2 and 8 h the heart rate reduction following salbutamol 8 mg was significantly less than the comparable fall after placebo. ICI 118,551 alone and salbutamol plus ICI 118,551 were not different from placebo.

Time-domain methods (Table 1: Figure 3)

There were significant between treatment differences for actions on the long (SDNN, SDANN) and short-term

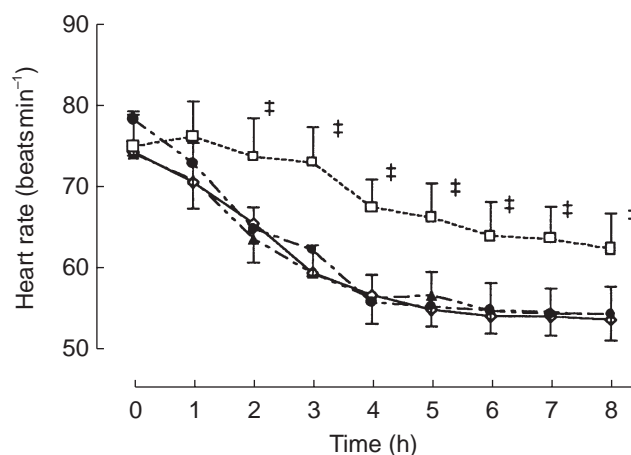


Figure 2 Effects on heart rate (over 8 h) following placebo (▲), salbutamol 8 mg (□) ICI 118,551 25 mg (◇) and salbutamol 8 mg plus ICI 118,551 25 mg (●). Statistics related to between group comparisons: non-parametric ANOVA followed by Wilcoxon's rank sign test. ‡*P*<0.01 vs salbutamol. Data are mean ± 95% confidence interval.

(rmsSD) HRV summary statistics. The long-term measurements SDNN and SDANN were significantly reduced (vs placebo) by salbutamol 8 mg, unaffected by ICI 118,551, and the reduction in HRV attributable to salbutamol was prevented when given in combination with ICI 118,551.

The short-term HRV parameters of rmsSD and pNN50 were significantly reduced (vs placebo) by salbutamol 8 mg; ICI 118,551 alone had no effect but when given in combination with salbutamol, it prevented the fall in these short-term HRV parameters.

Effects on scatterplot parameters (Table 1: Figures 4, 5)

A significant reduction in scatterplot length and area (Figure 4) followed salbutamol 8 mg compared with placebo, ICI 118,551 alone did not affect scatterplot

Table 1 Mean values [95% confidence intervals] for HRV variables.

Variable	Salbutamol	Placebo	ICI 118,551	Salbutamol + ICI 118,551
Heart rate (beats min ⁻¹)	67 [64, 71]	-9 [-13, -6]‡	-10 [-13, -7]‡	-10 [-12, -7]‡
rmsSD (ms)	51 [42, 60]	12 [5, 20]‡	16 [9, 23]‡	14 [8, 21]‡
pNN ₅₀ (ms)	30 [22, 37]	10 [3, 18]‡	13 [7, 20]‡	12 [6, 19]‡
SDNN (ms)	138 [120, 156]	39 [24, 55]‡	42 [23, 61]‡	44 [25, 62]‡
SDANN (ms)	107 [89, 124]	42 [29, 56]‡	37 [19, 55]‡	42 [24, 60]‡
Scatterplot area*10 ³ (ms ²)	222 [191, 253]	59 [36, 83]‡	57 [30, 84]‡	34 [28, 81]‡
Scatterplot length (ms)	944 [869, 1019]	164 [98, 230]‡	187 [118, 256]‡	157 [96, 219]‡
RR-1 75% width (ms)	417 [391, 443]	41 [20, 62]‡	38 [21, 55]‡	28 [5, 51]‡

Statistics (Non-parametric ANOVA with Friedman's comparison procedure: †*P*<0.05 vs salbutamol; ‡*P*<0.01 vs salbutamol). Values for salbutamol are expressed as mean [95% confidence intervals]; other columns express mean difference from salbutamol [95% confidence intervals for difference]. Data analyses were over 8 h (00.00–07.59 h).

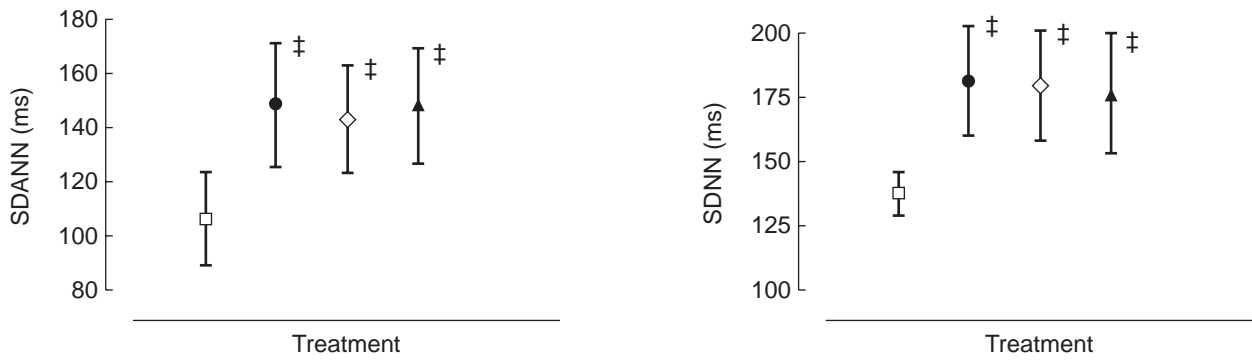


Figure 3 Effects on SDNN and SDANN (over 8 h) following placebo (▲), salbutamol 8 mg (□), ICI 118,551 25 mg (◇) and salbutamol 8 mg plus ICI 118,551 25 mg (●). Statistics related to between group comparisons: non-parametric ANOVA followed by Wilcoxon's rank sign test. ‡ $P < 0.01$ vs salbutamol. Data are mean \pm 95% confidence interval.

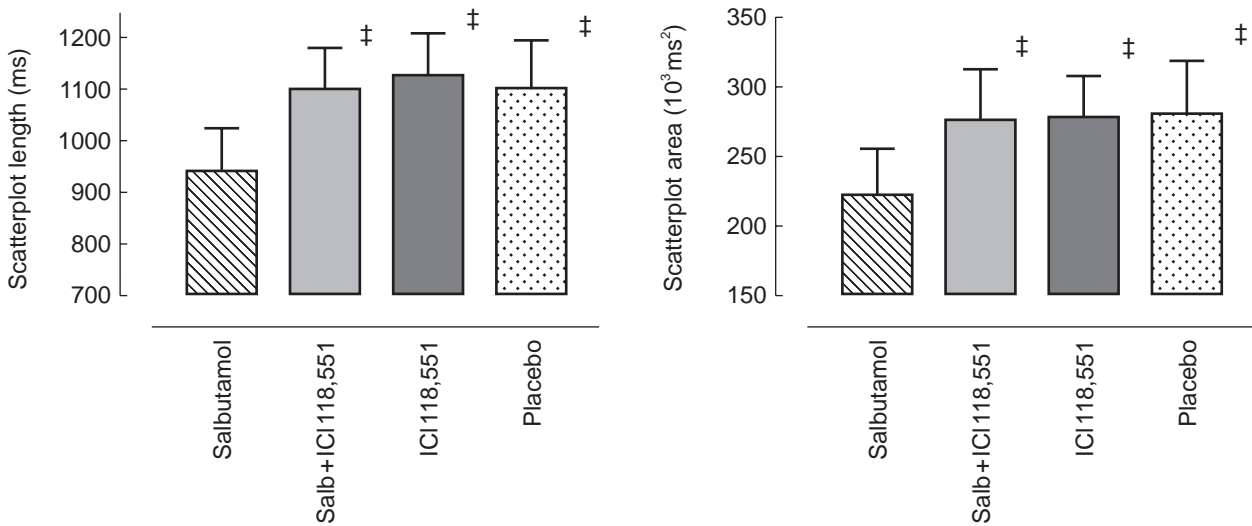


Figure 4 Effects on scatterplot length and area (over 8 h) following placebo, salbutamol 8 mg, ICI 118,551 25 mg and salbutamol 8 mg plus ICI 118,551 25 mg. Statistics related to between group comparisons: non-parametric ANOVA followed by Wilcoxon's rank sign test. ‡ $P < 0.01$ vs salbutamol. Data are mean \pm 95% confidence interval.

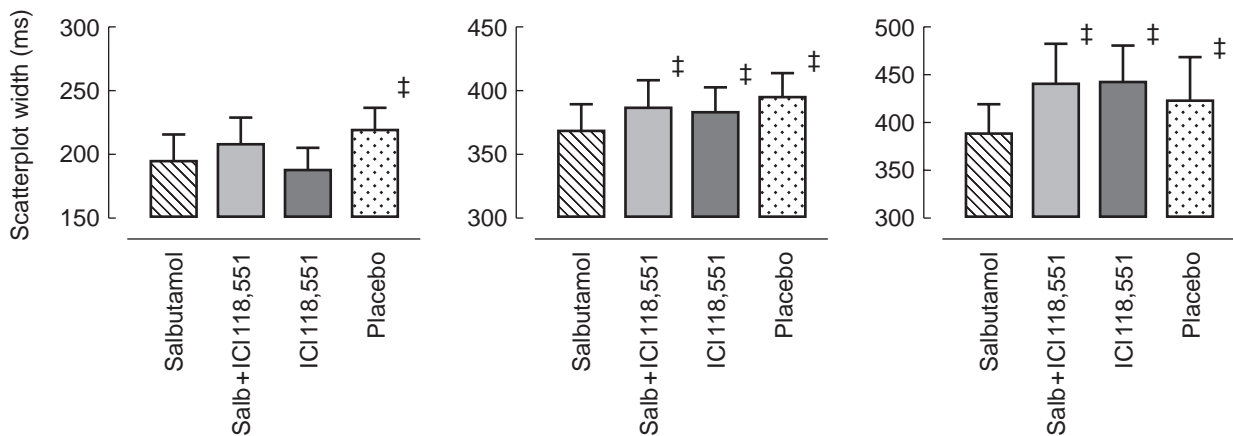


Figure 5 Effects on scatterplot width (over 8 h) at the 10th, 50th and 90th percentile following placebo, salbutamol 8 mg, ICI 118,551 25 mg and salbutamol 8 mg plus ICI 118,551 25 mg. Statistics related to between group comparisons: non-parametric ANOVA followed by Wilcoxon's rank sign test. ‡ $P < 0.01$ vs salbutamol. Data are mean \pm 95% confidence interval.

length or area but in combination with salbutamol prevented the reduction in these scatterplot indices.

The geometric analysis of the scatterplots allowed assessment of HRV effects at fixed RR intervals. Differences between the drugs (Figure 5) were present over the entire scatterplot length. Salbutamol 8 mg significantly reduced the width of the scatterplot across the whole range (P10%, P25%, P50%, P75%, and P90%) compared with placebo. ICI 118,551 alone had no effect on scatterplot widths but prevented the actions of salbutamol when given in combination.

Quadrant analysis: effect of treatment on total counts (Figure 6)

Analysis of each quadrant for total counts over 7 hr (00.00–06.59 h), considered in turn intervals of type +/+ (a), +/- (b), -/+ (c), or -/- (d); salbutamol shifted the autonomic balance towards sympathetic dominance. Cardiac acceleration episodes (i.e. no. of times ΔRR and ΔRR_{n+1} were both altered in the same direction) were increased in quadrant a and d following salbutamol. In quadrant d for example (Figure 6), the count frequency increased after salbutamol 8 mg compared with placebo, ICI 118,551 25 mg alone had no effect, but in combination blocked the shift attributable to salbutamol. The absolute magnitude of the sequence difference was also determined. Salbutamol 8 mg reduced the average duration of the beat-to-beat difference; for quadrant d for example, the bias towards cardiac acceleration was reflected in the shortened beat-to-beat difference (ΔRR_{n+1}) compared with placebo, ICI 118,551 or ICI 118,551 plus salbutamol 8 mg (38 [31,

44] vs 44 [38, 50], 47 [40, 54] and 45 [37, 53] ms respectively; $P < 0.01$ for each comparison).

Discussion

Although many studies have considered the impact of the β -adrenoceptor group on autonomic balance and the regulation of HRV, the relative role and importance of the β -adrenoceptor subtypes in the modulation of HRV is not established. The interaction between receptor subtype and stimulation or blockade of the β -adrenoceptor has received limited consideration, although it may be relevant to secondary prevention goals after myocardial infarction [6, 7]. We specifically considered the relevance of agonism and antagonism at the β_2 -adrenoceptor. We felt that comparison of the β_2 agonist effects of salbutamol and the antagonist effects of the highly β_2 -selective adrenoceptor antagonist ICI 118,551 on HRV would be instructive in this regard. Salbutamol has, depending on the model used, either partial or full agonist activity at the β_2 -adrenoceptor [22, 30]; its chronotropic actions are consistent with effects at the cardiac β_2 -adrenoceptor [31, 32]. On the other hand, ICI 118,551 possessed a high degree of selectivity and specificity for the β_2 -adrenoceptor; it caused a dose-dependent parallel shift to the right of the isoprenaline vasodilator curve without alteration of the corresponding isoprenaline chronotropic curve. Additionally ICI 118,551 possessed at least a 250 fold greater affinity for the vascular β_2 -adrenoceptor than the cardiac β_1 -adrenoceptor [24]. Thus it represented a useful drug for examining the role of the β_2 -adrenoceptor in physiological control mechanisms and in disease states. The dose of ICI 118,551 used in this study had no or

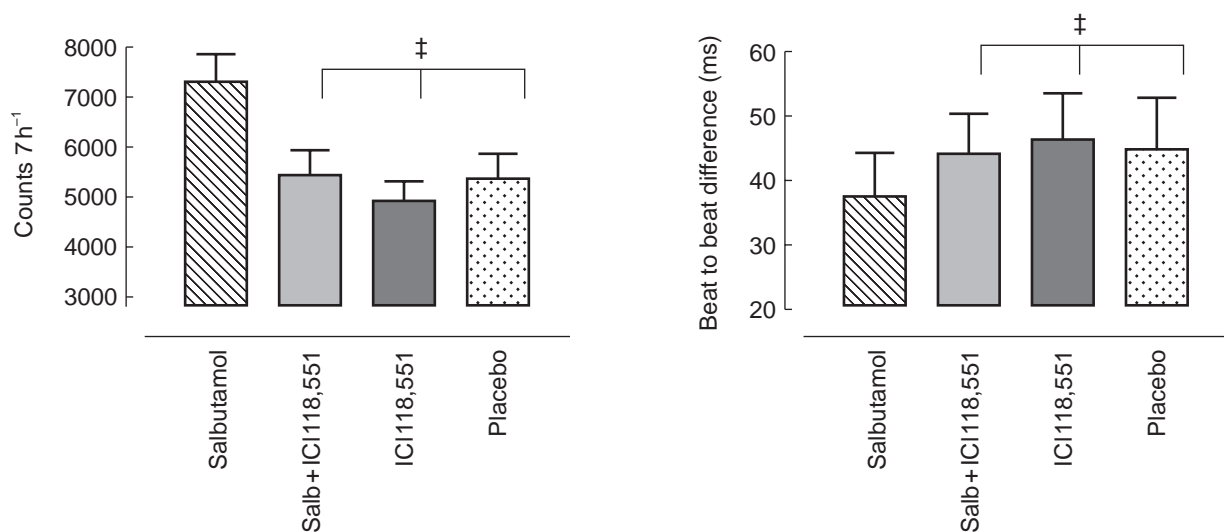


Figure 6 Effects on quadrant -/- (d: acceleration/acceleration) (over 7 h) counts and mean interval difference following placebo, salbutamol 8 mg, ICI 118,551 25 mg and salbutamol 8 mg plus ICI 118,551 25 mg. Statistics related to between group comparisons: non-parametric ANOVA followed by Wilcoxon's rank sign test. ‡ $P < 0.01$, all three vs salbutamol. Data are mean \pm 95% confidence interval.

minimal effects on the β_1 -adrenoceptor [20, 25] or on the sleeping heart rate [19].

Our analyses suggest a role for the β_2 -adrenoceptor in the modulation of HRV; the variability was reduced by the β_2 -adrenoceptor agonist salbutamol in contrast to β_2 -adrenoceptor antagonism with ICI 118,551, that was devoid of any such action. However when ICI 118,551 was administered together with salbutamol, the reductions in HRV parameters, induced by salbutamol alone, were abolished. Therefore stimulation of the β_2 -adrenoceptor increased HR and reduced HRV; however blockade of the β_2 -adrenoceptor did not by itself alter HR or HRV.

At apparent variance with these findings is the literature data, that have largely suggested improved HRV parameters following β -adrenoceptor blockade. However most studies have utilised β_1 -adrenoceptor antagonists; the implications for HRV of agonism at a receptor have received little emphasis. Cook *et al.* [33] demonstrated that atenolol in normal volunteers improved time and frequency HRV measures; measures of tonic vagal activity rmsSD, pNN50 and high frequency power increased by 61%, 69% and 84% respectively. Schweizer *et al.* [34], also in normal volunteers, found that metoprolol 100 mg significantly lowered power spectral density in the low frequency band (0.03 Hz to 0.15 Hz) and concluded that this agent inhibited cardiac sympathetic activation. Niemela *et al.* [35] reported that β -adrenoceptor blockade, with 200 mg controlled release metoprolol or atenolol 100 mg for 2 weeks in 18 patients with stable coronary disease, similarly increased heart rate variability; the average 24-h high frequency power (atenolol +64%; metoprolol +62%), the rmsSD (atenolol +70%; metoprolol +62%) and SDRR (atenolol +20%; metoprolol +16%) were increased. Sandrone *et al.* [9], with spectral techniques, demonstrated that atenolol or metoprolol reduced sympathetic activation and increased vagal tone in 20 patients, 4 weeks after the first uncomplicated myocardial infarction. Atenolol and metoprolol exerted more pronounced effects in the daytime compared with the night; there was also a marked attenuation in the circadian variation of the low-frequency component after each drug.

Hohnloser *et al.* [36] investigated HRV, assessed by time- and frequency-domain measures, after sotalol in 28 patients with chronic ventricular arrhythmia. Therapy with sotalol produced a significant improvement in indices of parasympathetic tone (SDNN, rmsSD, pNN50 and high-frequency power spectrum). Other studies in patients with congestive heart failure have shown that 2 months treatment with the β_1 -selective adrenoceptor antagonist, bisoprolol (5 mg day⁻¹), have increased rMSSD, pNN₅₀, SDNN and high frequency spectral power during the daytime [28]; metoprolol has also improved short-term time domain HRV variables in

coronary disease [37, 38]. Taken together this substantial body of evidence suggests that antagonism at the β_1 -adrenoceptor increased time and frequency measures of HRV. These findings are consistent with a shift in autonomic balance, representing an enhanced parasympathetic and attenuated sympathetic state.

In contrast, Stein *et al.* [39] determined the effects of pindolol (5 mg twice daily) and demonstrated a reduced HRV in normal subjects. Pindolol (agonist activity predominately at the β_2 -adrenoceptor [40]) reduced the HR extremes and decreased SDNN, SDANN, ultra-low frequency and total spectral power. It was only with the reduced sympathetic drive at night that pindolol's effects in reducing the time domain indices of HRV (SDNN, SDANN, rMSSD and pNN₅₀) were apparent. Consistent with this finding, that agonist activity at the β_2 -adrenoceptor with pindolol reduced HRV, was that salbutamol, a β_2 -selective adrenoceptor agonist, shifted cardiovascular autonomic balance towards sympathetic dominance and decreased cardio-vagal nervous responsiveness [41]. Another selective β_2 -adrenoceptor agonist, terbutaline, also decreased the RR interval variability indices related to vagal tone; the parasympathetic nervous reactivity decreased [42]. Celiprolol (dose-dependent β_2 -adrenoceptor partial agonist [43]) reduced short and long-term time domain statistics together with scatterplot length, area and width [11]. In addition sequence analysis demonstrated an increased frequency of acceleration episodes, in accord with the hypothesis of a shift from balanced autonomic control to sympathetic dominance. These effects of celiprolol (800 mg), in reducing HRV indices, were attenuated by β_1 - and β_2 -adrenoceptor blockade with propranolol 160 mg but not by β_1 -adrenoceptor blockade with 50 mg atenolol [11]. Therefore one can conclude that agonism at the β_2 -adrenoceptor reduces HRV in contrast to antagonism at the β_1 -adrenoceptor, when HRV indices are increased. In the present study antagonism at the β_2 -adrenoceptor with ICI 118,551 did not increase HRV; however the lack of any HRV effect could merely reflect the absence of tonic tone at the β_2 -adrenoceptor at night. Whether agonist activity at the β_1 -adrenoceptor would similarly reduce HRV and the relative importance of the β_1 - and β_2 -adrenoceptors in the modulation of HRV cannot be inferred from the available evidence.

The optimum method to investigate heart rate variability during drug intervention is uncertain. The standard time-domain methods include the SDNN, reflecting all cyclic components responsible for variability in the recording, whereas the SDANN and SDNN index represent variability over cycles longer and shorter than 5 min respectively. Non-linear methods have been suggested as an alternative approach, the disadvantages of the time-domain based methods namely the strong

correlation between adjacent beats, can be to some extent circumvented. The geometric scatterplot method investigates beat-to-beat variations in heart rate using a visual or pattern recognition approach; it is based on non-linear considerations and assesses non-periodic fluctuations in the heart rate. The scatterplot (Poincaré plot) had independent prognostic value for all-cause and sudden death in heart failure [44]. Human research investigations have correlated scatterplot length with long-term variability indices [17] whereas scatterplot width was correlated with short-term HRV and parasympathetic nervous activity [17, 18, 38]. The impact of β -adrenoceptor antagonism on scatterplot measures of HRV is not established; in patients with chronic heart failure bisoprolol did not alter scatterplot length but increased width at the highest percentiles (lowest heart rates) [28]. However metoprolol, in survivors of myocardial infarction, increased time-domain HRV statistics (mean RR interval, rMSSD, and proportion of adjacent RR intervals differing by >50 ms [pNN50 (%)]) at unaltered scatterplot indices [38]. It should be appreciated that the conventional scatterplot does not take into account the density of the paired RR intervals; the same two-dimensional shape may therefore reflect different patterns of HRV. A numeric quantitation of 3D scatterplots has been suggested where the third co-ordinate corresponds to the number of observations at discrete points along the scatterplot length; this approach improves the predictive accuracy of the method [16].

The other non-linear approach, used in this paper, was the ΔRR vs ΔRR_{n+1} analysis; this method of sequence analysis also removes the dominant characteristic of the strong correlation between consecutive intervals present in both the time-domain and scatterplot analyses. Therefore the time-dependent dynamics of HRV may be assessed. Perturbations of autonomic balance, would be expected to alter the likelihood of the occurrence of particular sequences of acceleration ($-/-$) or deceleration ($+/+$), reflecting the shift in balance towards sympathetic or parasympathetic predominance. The normal pattern of the ΔRR vs ΔRR_{n+1} analysis in the 'awake' state is alternation of increases and decreases in interval length, contrasting with 'quiet' sleep where sequences of progressively increasing and decreasing intervals occur [13]. Other suggested non-linear measurements include the approximate entropy and fractal dimension [42]; an advantage of these methods is that the prerequisites of data stationary and sinusoidal nature required for spectral analysis are not so stringent nor are the parameter estimates so strongly correlated with the mean.

The data from these disparate analyses was consistent, whether the HRV estimates were derived from the time-domain, the scatterplot or quadrant analyses. The main

disadvantage of the traditional time-domain summary statistics is the strong dependence on the mean heart rate; for agonists and antagonists of the cardiac β -adrenoceptor, it would be preferable for there to be a rate independent measure of variability. The scatterplot analysis provides parameters that can be interrogated at fixed heart rates [28], while the sequence methods is more highly de-correlated from the mean [14]. In purely statistical terms, in the present assessment none of the methods was of proven superiority; although measuring the differing aspects of HRV, all gave a similar estimate of the pharmacodynamic effects.

In conclusion, the non-linear methods of scatterplot and quadrant analysis permitted autonomic balance to be assessed following therapeutic intervention with agents that modulated the β -adrenoceptor in man. The newer methods were at least as robust as the available time-domain methods. Partial agonism with salbutamol 8 mg altered the autonomic balance towards sympathetic dominance; ICI 118,551 25 mg had no effect on HRV but prevented the HRV reduction seen when salbutamol was given alone. β -adrenoceptor antagonists have improved survival following myocardial infarction [6, 7]; whether the observed effect of a partial agonist, with decreased parasympathetic and enhanced sympathetic responsiveness, would offset the cardio-protective benefits of β -adrenoceptor blockade in secondary prevention is unknown. However these studies highlight such questions and suggest a direction for further research.

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